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BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application Number: 09/287,632
Filing Date: April 07, 1999
Appellant(s): WATERHOUSE ET AL.

Christopher L. North
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed April 8, 2010 appealing from the Office action mailed December 8, 2009.

(1) Real Party in Interest

The examiner has no comment on the statement, or lack of statement, identifying by name the real party in interest in the brief.

(2) Related Appeals and Interferences

The examiner is not aware of any related appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

(3) Status of Claims

The following is a list of claims that are rejected and pending in the application:

Claims 1-10, 12, 22, 26, 40, 42-44, 46, 50, 53, 54, 56, 58, 63-69, 98-103, 109, 111-122 are pending in the instant application. Claims 22, 26, 42, 53, 54, 56, 58, 63-69, 100-103, 109, 115-122 stand rejected and are the subject of this appeal. Claims 1-10, 12, 40, 43, 44, 46, 50, 98, 99, 111-114 have been withdrawn from consideration pursuant to a restriction requirement.

(4) Status of Amendments After Final

The examiner has no comment on the appellant's statement of the status of amendments after final rejection contained in the brief.

(5) Summary of Claimed Subject Matter

The examiner has no comment on the summary of claimed subject matter contained in the brief.

(6) Grounds of Rejection to be Reviewed on Appeal

The examiner has no comment on the appellant's statement of the grounds of rejection to be reviewed on appeal. Every ground of rejection set forth in the Office action from which the appeal is taken (as modified by any advisory actions) is being maintained by the examiner except for the grounds of rejection (if any) listed under the

subheading "WITHDRAWN REJECTIONS." New grounds of rejection (if any) are provided under the subheading "NEW GROUNDS OF REJECTION."

NEW GROUND(S) OF REJECTION

There are no new grounds of rejection.

WITHDRAWN REJECTIONS

There are no withdrawn rejections.

(7) Claims Appendix

The examiner has no comment on the copy of the appealed claims contained in the Appendix to the appellant's brief.

(8) Evidence Relied Upon

6,506,559	Fire et al.	January - 2003
5,859,347	Brown et al.	January - 1999
6,350,575	Lusky et al.	February - 2002
5,801,154	Baracchini et al.	September - 1998

Schiedner et al., *Genomic DNA transfer with a high-capacity adenovirus vector results in improved in vivo gene expression and decreased toxicity*, Nature Genetics, Vol. 18, pages 180-183 (1998).

Flavell et al., *Inactivation of gene expression in plants as a consequence of specific sequence duplication*, Proc. Natl. Acad. Sci., Vol. 91, pages 3490-3496 (1994).

Metzlaff et al., *RNA-mediated RNA degradation and chalcone synthase A silencing in petunia*, Cell, Vol. 88, pages 845-854 (1997).

Stam et al., *The silence of genes in transgenic plants*, Annals of Botany, Vol. 79, pages 3-12 (1997)

Nobelprize.org: The Nobel Prize in Physiology or Medicine 2006, Press Release of the Nobel Assembly at Karolinska Institute (2 October 2006).

(9) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

to 37 CFR 1.114.

Claims 22, 26, 42, 53, 54, 56, 58, 63-69, 100-103, 109, and 115-122 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that

the inventor(s), at the time the application was filed, had possession of the claimed invention for the reasons of record set forth in the Office action mailed 12-8-09.

The claims are drawn to any plants and any eukaryotic cells comprising any nucleic acid of interest which is capable of being phenotypically expressed, and comprising any chimeric DNA comprising an operable promoter, transcription termination and polyadenylation region, and further comprising a DNA region encoding a region capable of forming an artificial hairpin RNA structure with a double stranded RNA stem by base pairing between regions with a sense and an antisense nucleotide sequence, which sense nucleotide sequence includes at least 20, 50, 100, or 550 consecutive nucleotides having 100% sequence identity with at least 20, 50, 100, or 550 consecutive nucleotides of any nucleic acid of interest, and which antisense sequence includes at least 20, 50, 100, or 550 consecutive nucleotides having 100% sequence identity with said at least 20, 50, 100, or 550 consecutive nucleotides of the sense sequence, and which chimeric DNA further comprises an intron.

The specification fails to teach or adequately describe a representative number of species in the genus such that the common attributes or characteristics concisely identifying members of the proposed genus are exemplified (e.g. the myriad of sequences encompassed by the genus intron, or intronic sequences is vast, and further whereby any intronic sequence is inserted anywhere within the DNA construct and a DNA chimeric construct generates a gene silencing construct which reduces the phenotypic expression of any nucleic acid of interest in any eukaryotic cell). And because the genus claimed is so highly variant, the description provided is insufficient

whereby a representative number of chimeric constructs provide for the functions claimed, of reducing the phenotypic expression of any nucleic acid of interest in any eukaryotic cell or plant. One of skill in the art would reasonably conclude that the disclosure, at the time of filing, fails to provide a representative number of species to describe the broad genus claimed.

The instant rejection is directed to eukaryotic cells and plants comprising chimeric nucleic acids comprising RNAi constructs that comprise lengths as small as 20 nucleotides that are fully complementary and target a nucleic acid capable of being phenotypically expressed. There is no teaching, prior to Fire's disclosure, of the inhibition of target gene expression using double stranded inhibitory constructs of less than approximately 550 base pairs. Applicants did not have support, prior to Fire's disclosure, for the limitations instantly claimed, particularly with respect to siRNA of lengths as short as 20 nucleotides in length per strand.

The initial disclosure teaches fully complementary pair constructs for reducing the phenotypic expression of a transgenic Gus gene (of approximately 1580 base pairs), and complementary pair constructs for reducing the phenotypic expression of the Δ12 desaturase target gene in *Arabidopsis* (of approximately 620 base pairs) which complementary pair constructs additionally comprise the pyruvate orthophosphate dikinase 2 intron 2 from *Flaveria trinervia* (SEQ ID NO. 7) in forward or reverse orientation. The declarations filed 5-1-08 teach three expression constructs, one which comprises a double stranded hairpin of at least 750 base pairs that comprises a sequence fully complementary to and targets the potato virus Y gene, and which

expression construct further comprises intron 2 of pdkA, which was transformed into *Agrobacterium*, and a second and third double stranded construct of at least 558 base pairs that targets and is fully complementary to the target GUS gene, and optionally comprise the Ubi-1 intron, which nucleic acid constructs were transformed into rice calli.

The examples provided in the declarations and in the instant disclosure are not representative of the very broad genus of compounds, eukaryotic cells, and plants claimed, which encompass any plant and any eukaryotic cell comprising any nucleic acid capable of expressing a phenotype, and comprising chimeric DNA comprising an operable promoter, transcription termination and polyadenylation region, and further comprising any DNA region encoding a region capable of forming an artificial hairpin RNA structure with a double stranded RNA stem by base pairing between regions with a sense and an antisense nucleotide sequence, which sense nucleotide sequence includes at least 20, 50, 100, or 550 consecutive nucleotides having 100% sequence identity with at least 20, 50, 100, or 550 consecutive nucleotides of any nucleic acid of interest, and which antisense sequence includes at least 20, 50, 100, or 550 consecutive nucleotides having 100% sequence identity with said at least 20, 50, 100, or 550 consecutive nucleotides of the sense sequence, and which chimeric DNA further comprises an intron.

Applicants, by providing the examples described above, were not in possession of the broad array of compounds, cells, or plants claimed, comprising any nucleic acid capable of expressing a phenotype, and further comprising a hairpin construct fully complementary and targeting any nucleic acid capable of expressing a phenotype, at

the time of filing. The genus of plants, eukaryotic cells and chimeric constructs is expansive, and adequate representation of species encompassing this myriad of plants, eukaryotic cells and chimeric constructs has not been made, either prior to December 23, 1997, or at the time of filing.

See also page 3 of the declaration of Marc De Block submitted on 6-8-07, paragraph 14, reporting hairpin constructs that failed to provide a predictable phenotype of differences in flowering in oilseed rape, and depended on the insertion of a particular intron in a particular expression construct. The declarations and experimental examples provided in the initial disclosure failed to teach the successful inhibition using RNAi molecules with size ranges of less than approximately 550 nucleotides per targeting strand. No examples of less than 550 nucleotides per targeting strand were provided by Applicant.

Thus, Applicant was not in possession of the broadly claimed genus.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein

were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 22, 26, 42, 53, 54, 56, 58, 63-69, 100-103, 109, 115-122 are rejected under 35 U.S.C. 103(a) as being unpatentable over Fire et al (USPN 6,506,559) in view of Brown et al (USPN 5,859,347), Lusky et al (USPN 6,350,575) and Schiedner et al (Nature: Genetics, Vol. 18, pages 180-183, 1998), the combination in view of Baracchini et al (USPN 5,801,154) insofar as the claims are drawn to plants, eukaryotic cells, and chimeric DNA comprising an operable promoter, transcription termination and polyadenylation region, and further comprising a DNA region encoding a region capable of forming an artificial hairpin RNA structure with a double stranded RNA stem by base pairing between regions with a sense and an antisense nucleotide sequence, which sense nucleotide sequence includes at least 20 consecutive nucleotides having 100% sequence identity with at least 20 consecutive nucleotides of a nucleic acid of interest, and which antisense sequence includes at least 20 consecutive nucleotides having 100% sequence identity with said at least 20 consecutive nucleotides of the sense sequence, and which chimeric DNA further comprises any intronic sequence for the reasons of record set forth in the Office action mailed 12-8-09.

Applicant has not provided proper support for the size limitations instantly claimed to exclude Fire as prior art. Applicant provided declarations on 5-1-08

describing experiments that were performed prior to December 23, 1997, the priority date of the Fire patent, USPN 6,506,559, to provide support for being awarded a priority date prior to December 23, 1997 for the instant claims. The instant rejection, however, is directed to eukaryotic cells and plants comprising chimeric nucleic acids comprising RNAi constructs that comprise at least 20 nucleotides that are fully complementary and target a nucleic acid capable of being phenotypically expressed. Applicant has not provided proper support for the short nucleobase size limitations of the instant claims, which are drawn to RNAi constructs comprising at least 20 nucleotides in length. Support has been provided for double stranded constructs comprising more than 550 nucleotides in length, but not comprising 20 nucleotides in length. For these reasons, the prior art of Fire still stands as valid prior art.

The instant specification teaches fully complementary pair constructs for reducing the phenotypic expression of a transgenic Gus gene (of approximately 1580 base pairs), and complementary pair constructs for reducing the phenotypic expression of the Δ12 desaturase target gene in *Arabidopsis* (of approximately 620 base pairs) which complementary pair constructs additionally comprise the pyruvate orthophosphate dikinase 2 intron 2 from *Flaveria trinervia* (SEQ ID NO. 7) in forward or reverse orientation. The declarations filed 5-1-08 teach three expression constructs, one which comprises a double stranded hairpin of at least 750 base pairs that comprises a sequence fully complementary to and targets the potato virus Y gene, and which expression construct further comprises intron 2 of pdkA, which was transformed into *Agrobacterium*, and a second and third double stranded construct of at least 558

base pairs that targets and is fully complementary to the target GUS gene, and optionally comprise the Ubi-1 intron, which nucleic acid constructs were transformed into rice calli.

Fire et al (USPN 6,506,559) (*hereinafter* "Fire") teach plant cells, plants and their seeds comprising a nucleic acid comprising a first and second DNA sequence which expresses in the plant cell a chimeric DNA comprising a promoter, operatively linked to a DNA region which, when transcribed, yields and RNA molecule capable of forming a hairpin comprising two annealing RNA sequences which comprise a sense sequence sharing homology with consecutive nucleotides of a target nucleic acid of interest in the plant, and which further comprises a second, annealing RNA sequence comprising antisense sharing homology with the consecutive nucleotides of the sense strand that targets the nucleic acid of interest, and which chimeric DNA further comprises operably linked transcription termination and polyadenylation sequences (see esp. claims 10, 15) (See also the abstract, col. 3-4, col. 5, line 47-col. 6, line 54, col. 7, line 42-col. 9, line 25, col. 11, line 37-col. 12, line 8, col. 17, line 20-24, col. 21, line 36-col. 22, line 4; col. 4, lines 41-61; col. 6, line 32-col. 9, line 48; col. 12, lines 46-col. 13, line 8; claims 1-12 and 21).

As for a hairpin structure between the sense and antisense strands of the siRNA, Fire explicitly teaches in lines 43-47 of col. 4 (emphasis added):

> The double-stranded structure may be formed **by a single self-complementary RNA strand or two complementary RNA strands**. RNA duplex formation may be initiated either inside or outside the cell…

Fire reiterates this in col. 7, lines 42-45. In addition, in col. 13, lines 61-63, Fire teaches that such siRNA constructs may include "the RNA molecule itself, an **expression construct** capable of expression the RNA, or organisms transfected with the expression construct." And, regarding the components to be optionally included within a recombinant expression construct for expressing the siRNA, Fire also explicitly teaches in the bridging paragraph, col. 8-9 (emphasis added) (citations omitted):

> RNA may be synthesized either in vivo or in vitro. Endogenous RNA polymerase of the cell may mediate transcription in vivo, or cloned RNA polymerase can be used for transcription in vivo or in vitro. For transcription from a transgene in vivo or an expression construct, a regulatory region (**e.g.,** promoter, enhancer, silencer, splice donor and acceptor, polyadenylation) may be used to transcribe the RNA strand (or strands)… The RNA strands may or may not be polyadenylated; the RNA strands may or may not be capable of being translated into a polypeptide by a cell's translational apparatus... The use and production of an expression construct are known in the art...

Fire does not teach explicitly the RNAi expression constructs comprising an intervening intron sequence.

Brown et al (USPN 5,859,347) (*hereinafter* "Brown") teach plant cells transformed with chimeric nucleic acid expression constructs expressing desired DNA sequences, and which expression constructs comprise expression elements including operably linked promoters and further comprising heterologous introns, which introns enhance stability and expression of the nucleic acid sequences in an expression construct (see col. 8, line 53-col. 9, line 17, examples 1-7 in cols. 10-18 and figures 8-27).

Lusky et al (USPN 6,350,575) (*hereinafter* "Lusky") teach expression constructs comprising antisense RNA and further comprising an intron as well as other expression elements including translation termination and polyadenylation signals (col. 6, line 15-col. 7, line 14).

Schiedner et al (Nature: Genetics, Vol. 18, pages 180-183, 1998) (*hereinafter* "Schiedner") teach expression vectors comprising intronic sequences for enhancing vector stability (see esp. left col., p. 180).

Baracchini et al (USPN 5,801,154) (*hereinafter* "Baracchini") teach the motivation and ability to target a gene of interest with a complementary sequence comprising at least 10 nucleobases (see e.g. claims 1, 12, 26, and 32).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to design and utilize chimeric constructs as instantly claimed to alter the expression of a target gene of known sequence, which gene is either endogenous or heterologous to a plant cell, which target gene is either stably integrated or extrachromosomal, comprising the introduction of nucleic acids comprising sense and complementary antisense sequences of the target gene, which are operably linked to a constitutive or heterologous promoter, and which are optionally expressed on separate or the same expression construct, and hybridize after their expression to the complementary sequences of each other to form a double stranded molecule, whereby a duplex is formed between the expressed sense and antisense fragments, because the efficiency of such methods of gene silencing have been previously taught Fire. One of ordinary skill in the art would have constructed RNAi constructs comprising 25

nucleobases on the sense and on the antisense strand, which are fully complementary to the target nucleic acid of interest because Fire discloses RNAi molecules of this short size range. One of ordinary skill in the art would have expected the expressed double stranded RNA to target and inhibit the expression of corresponding target sequences of a target gene of known sequence, as taught previously by Fire.

One of ordinary skill in the art would have been motivated to include intronic sequences within the expression constructs for gene expression in plants because the use of intronic sequences for enhancing vector stability and hence enhance expression of a desired gene in cells had been taught previously by Brown and Schiedner. What's more, Lusky and Fire also teach the incorporation of intronic sequences in expression constructs and it was well known in the art that the inclusion of introns enhances the expression of RNA in plants. One of ordinary skill in the art would have optionally placed the intronic sequences between the sense and antisense sequences in the chimeric construct originally taught by Fire because this is a design choice and additional, non-complementary sequences (e.g. intronic sequences) are included in the sense antisense constructs in order to allow for hairpin turns between complementary sequences.

One of ordinary skill in the art would have expected that the intronic sequences, inserted at different places in the expression construct, would enhance expression of the chimeric constructs in plants and it would take routine experimentation to determine where in the construct the intron sequences would be inserted, as long as complementarity between the sense and antisense sequences was maintained for

subsequent target gene inhibition. One of ordinary skill in the art would have been motivated to include the size range of 25 nucleobases for the targeting strand of the double stranded gene silencing construct because Fire is the first to disclose siRNA constructs with targeting sequences to be in that size range (e.g. 25 nucleobases) and it would take routine experimentation to vary the range of sequences of the gene silencing constructs originally taught by Fire. Likewise, one of ordinary skill in the art would have expected that the range of 10-50 nucleobases, and sharing 100% homology would be effective in gene silencing, because Fire was the first to disclose RNAi constructs of this size range, Fire compared the advantages of various inhibitory oligonucleotides, including RNAi, antisense and ribozymes in their ability to inhibit target gene expression, and successful gene targeting has been routinely provided using antisense with a minimum length of 10 nucleobases (see also Baracchini). It would therefore take routine experimentation to alter the length of the target sequence as well as the homology required for successful gene silencing in plant cells relying on the teaching of Fire and Baracchini. One would have been motivated to express downstream and operatively linked sequences in DNA expression vectors to subsequently duplex RNA in a cell to target and inhibit target gene expression.

One of ordinary skill in the art would have been motivated to inhibit the expression of target genes by these expressed RNAi molecules, as described previously by Fire, for altering cellular phenotypes in order to study gene function, or to study the role of various target genes by comparing cellular processes in the absence or presence of these target genes expression, or to inhibit a deleterious pathogenic gene

of an invading organism in a plant cell by inhibiting pathogenic target gene expression using this technique of gene silencing. One of ordinary skill in the art would have expected that the inclusion of intronic sequences would enhance expression construct stability because the inclusion of intronic sequences in expression constructs was routine in the art, as evidence by the inclusion of intronic sequences in commercial and other published expression constructs, at the time the invention was made.

One of ordinary skill in the art would have expected that the transformation of expression cassettes for target gene silencing in appropriate plant cells, whereby the concerted expression of both the sense and antisense fragments in appropriate target cells using appropriate promoters is obtained, leads to the formation of double stranded fragments directed to the target gene sequences in the transformed cells, and consequently interferes with the expression of the target gene, thereby producing inhibition of target gene expression, allowing a comparison of cellular phenotypes in the presence and absence of target gene inhibition, as taught previously by Fire.

Therefore the invention as a whole would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.

Claims 22, 26, 42, 53, 54, 56, 58, 63-69, 100-103, 109, 115-122 are rejected under 35 U.S.C. 103(a) as being unpatentable over Flavell (Proc. Natl. Acad. Sci., Vol. 91, pages 3490-3496, 1994) Metzlaff et al (Cell, Vol. 88, pages 845-854, 1997), and Stam et al (Annals of Botany, Vol. 79, pages 3-12, 1997), the combination in view of

Brown et al (USPN 5,859,347) and Lusky et al (USPN 6,350,575) for the reasons of record set forth in the Office action mailed 12-8-09.

The claims are drawn to plants, eukaryotic cells, and chimeric DNA comprising an operable promoter, transcription termination and polyadenylation region, and further comprising a DNA region encoding a region capable of forming an artificial hairpin RNA structure with a double stranded RNA stem by base pairing between regions with a sense and an antisense nucleotide sequence, which sense nucleotide sequence includes at least 20 consecutive nucleotides having 100% sequence identity with at least 20 consecutive nucleotides of a nucleic acid of interest, and which antisense sequence includes at least 20 consecutive nucleotides having 100% sequence identity with said at least 20 consecutive nucleotides of the sense sequence, and which chimeric DNA further comprises any intronic sequence.

Flavell (Proc. Natl. Acad. Sci., Vol. 91, pages 3490-3496, 1994) (*hereinafter* "Flavell") teaches plants, eukaryotic cells, and chimeric DNA comprising an operable promoter, transcription termination and polyadenylation region, and further comprising a DNA region encoding a region capable of forming a double stranded RNA stem by base pairing between regions with a sense and an antisense nucleotide sequence, which sense nucleotide sequence includes at least 10 consecutive nucleotides having 100% sequence identity with at least 10 consecutive nucleotides of a nucleic acid of interest, and which antisense sequence includes at least 10 consecutive nucleotides having

100% sequence identity with said at least 10 consecutive nucleotides of the sense sequence (see esp. the abstract and text on pp. 3490-3491).

Flavell addresses the limitations of existing hypotheses of the day that were used to explain the observed co-suppression, and ponders the relationship between the existence of inverted repeats as well as the possible accumulation of sense/antisense constructs and the subsequence degradation of mRNA. See Flavell on p. 3490: "Degraded RNA products from both genes were found in co-suppressed fruit, suggesting that RNA transcription is not inhibited and therefore loss of mature mRNA is due to posttranscriptional turnover." (last full paragraph on p. 3490). See also the first full paragraph on p. 3491 which dispels the role of methylation in target gene inhibition: "…suppression of activity was not correlated with methylation..."

The second full paragraph of Flavell on p. 3491 provides motivation to study the role of self-complementary sequences in target gene inhibition: "Ninety base pairs of homology in promoter sequences were sufficient to create a co-suppressed condition." Numerous investigators in the mid-nineties were investigating the role of self-complementary constructs that formed aberrantly - and not excluding inverted repeats - in target gene inhibition. These self complementary constructs that resulted from the expression of inverted repeats were not limited to antisense-mRNA duplexes leading to mRNA degradation, and the presence and role of inverted repeats, particularly in observed flowering patterns in petunia, was questioned and discussed repeatedly throughout the 1990's.

The first paragraph on p. 3495 of Flavell invites further experimentation beyond the four proposed hypotheses of the day purportedly explaining co-suppression:

> What is needed to evaluate the application of antisense RNA formation to the cause of down-regulation of homologous gene expression, in at least some examples of trans-inactivation by transgenes, is a much better understanding of how antisense RNA effects down-regulation of gene expression, measurements of antisense and sense RNA levels in the relevant cells and their nuclei before as well as after RNA degradation, and knowledge of the role of RNA-dependent RNA polymerase and of the ability of accumulated RNA products to feed back and interfere with transcription. It will also be important to discover the relationship between the mRNA turnover revealed by transgenes and endogenous posttranscriptional control systems that regulate mRNA turnover.

Metzlaff et al (Cell, Vol. 88, pages 845-854, 1997) (*hereinafter* "Metzlaff") teach plants, eukaryotic cells, and chimeric DNA comprising an operable promoter, transcription termination and polyadenylation region, and further comprising a DNA region encoding a region capable of forming a double stranded RNA by base pairing between regions with a sense and an antisense nucleotide sequence (see esp. fig. 1 and 2 on p. 846; Table 1 and text on p. 849; text on p. 850; fig. 7 on p. 852).

Metzlaff discusses the role of inverted repeats in target gene inhibition: On page 845, second full paragraph of the introduction: "Petunia plants are correlated with the number of transgenes and their arrangement in the genome." Metzlaff devotes considerable thought to the relationship between self-annealing sequences and resistance of those self-annealing structures to degradation (see e.g. figure 5), as well as arguing that co-suppression is not merely due to the presence of high levels of chsA RNA (see first full paragraph on page. 850). The involvement of RNA-RNA pairing in RNA turnover is questioned: "Such complementarity may have been selected as a

component of an RNA turnover control system by intra- or intermolecular RNA pairing." (second full paragraph from the end on page 852). And, reviewing Jorgensen's work, the role of inverted repeats in target gene suppression is questioned: "...whereas multiple copies of transgenes, and especially **inverted repeat** copies, enhance the probability of more extensive co-suppression and, in particular, co-suppression in leaves and stems." (second last paragraph of the discussion on p. 853) (emphasis added).

Stam et al (Annals of Botany, Vol. 79, pages 3-12, 1997) (*hereinafter* "Stam") teach plants, eukaryotic cells, and chimeric DNA comprising an operable promoter, transcription termination and polyadenylation region, and further comprising a DNA region encoding a region capable of forming an artificial hairpin RNA structure with a double stranded RNA stem by base pairing between regions with a sense and an antisense nucleotide sequence, which sense nucleotide sequence includes at least 10 consecutive nucleotides having 100% sequence identity with at least 10 consecutive nucleotides of a nucleic acid of interest, and which antisense sequence includes at least 10 consecutive nucleotides having 100% sequence identity with said at least 10 consecutive nucleotides of the sense sequence, (see esp. bridging paragraph on pp. 3-4; fig. 1 on p. 4; text on page 8; fig. 3 on p. 9).

Stam repeatedly stresses the importance of the existence of inverted repeats and their role in gene inhibition: See figure 1 on p. 4 and the bridging paragraph of pages 3-

4: "These T-DNAs can be arranged 'head-to-tail' as a direct repeat (DR), and 'head-to-head' or 'tail-to-tail' as an inverted repeat (IR). **Transgenes of T-DNAs that are organized as IRs often show low expression indicating that the genes are silenced to some degree**." (citations omitted, emphasis added). And on p. 8, last full paragraph: "There are at least two possibilities; the first is that a multicopy locus is prone to deliver the hypothetical aberrant RNA assumed to trigger the cytosolic RNA degradation machinery directly... One possibility is that it occurs as a result of ectopic DNA pairing between the transgene locus and the endogenous gene(s)... these transcripts may be intrinsically unstable and rapidly degraded, or may act as aberrant RNA causing the degradation of other homologous RNAs. Not all transgene loci may be able to pair ectopically with an endogenous gene. An essential property seems that they are repetitive. **Thus far, all the T-DNA loci that we have found to induce PTGS of *chs* contain two or more T-DNAs arranged as IRs**... There are no indications that the methylation status of the endogenous genes is changed." (citations omitted, emphasis added).

And in the first paragraph on p. 9, Stam again rules out methylation as a mechanism involved in target gene inhibition involving IRs:

> Observations with IRs and DRs in Drosophila, which lacks 5-methyl-cytidine in its DNA, indicate that repeats somehow interact with each other, leading to the formation of heterochromatin… By analyzing petunia transformants carrying CaMV-35S promoter-driven *chs* sense or antisense transgenes, Jorgensen et al. (1996) showed that the pattern of *chs* silencing in flowers correlated with the repetitiveness and organization of the transgenes in these plants. The pigmentation pattern caused by single-copy transgene inserts is mostly regular (junction type) whereas that by IRs is often complex and sometimes recognizable as the 'Cossack Dancer' pattern…"

The primary references of Flavell, Metzlaff, and Stam do not teach double stranded hairpin constructs in their inverted repeats, nor do they teach the insertion of an intron in their double stranded inhibitory constructs.

Brown et al (USPN 5,859,347) (*hereinafter* "Brown") teach plant cells transformed with chimeric nucleic acid expression constructs expressing desired DNA sequences, and which expression constructs comprise expression elements including operably linked promoters and further comprising heterologous introns, which introns enhance stability and expression of the nucleic acid sequences in an expression construct (see col. 8, line 53-col. 9, line 17, examples 1-7 in cols. 10-18 and figures 8-27).

Lusky et al (USPN 6,350,575) (*hereinafter* "Lusky") teach expression constructs comprising antisense RNA and further comprising an intron as well as other expression elements including translation termination and polyadenylation signals (col. 6, line 15-col. 7, line 14).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to alter the expression of a target gene of known sequence, which gene is either endogenous or heterologous to a plant cell, which target gene is either stably integrated or extrachromosomal, comprising the introduction of nucleic acids comprising sense and complementary antisense sequences of the target gene, which are operably linked to a constitutive or heterologous promoter, and which are optionally expressed on separate or the same expression construct, and hybridize after their expression in the cell to the complementary sequences of each other to form a double

stranded molecule, whereby a duplex is formed between the expressed sense and antisense fragments, because this approach to gene silencing had been proposed and studied previously by Flavell, Metzlaff et al and Stam et al.

It is clear that the role of inverted repeats, and other double stranded RNA structures were pondered by those looking for underlying mechanisms of target gene suppression in eukaryotes, as illustrated by the teachings of Flavell, Stam and Metzlaff. It therefore would have been obvious to design, construct and test the ability of nucleic acid constructs comprising double stranded RNA for their ability to inhibit the expression of a known target gene in plants or in eukaryotic cells in vitro at the time of the instant invention.

One of ordinary skill in the art would have reasonably expected the expressed double stranded RNA to target and inhibit the expression of corresponding target sequences of a target gene of known sequence, because the correlation between gene silencing and the presence of these self complementary nucleic acid sequences were taught previously by Flavell, Metzlaff et al and Stam et al. One or ordinary skill in the art would have been motivated to design inverted repeats as a single molecule to test its inhibitory capacity because expression of a single, contiguous self annealing construct would provide for more efficient self annealing compared to two separately expressed self annealing molecules, applying scientific logic to the teachings of Flavell, Metzlaff and Stam concerning the ability of inverted repeats and self annealing, complementary nucleic acids to provide for target gene suppression.

One of ordinary skill in the art would have been motivated to include intronic sequences within the expression constructs for gene expression in plants because the use of intronic sequences for enhancing vector stability and hence enhance expression of a desired gene in cells had been taught previously by Brown et al. Furthermore, Lusky et al also teaches the incorporation of intronic sequences in expression constructs and it was well known in the art that the inclusion of introns enhances the expression of RNA in plants. One of ordinary skill in the art would have optionally placed the intronic sequences between the sense and antisense sequences in the inhibitory, double stranded, self complementary constructs originally taught Flavell, Metzlaff and Stam because this is a design choice. One of ordinary skill in the art would have expected that the intronic sequences, inserted at different places in the expression construct, would enhance expression of the chimeric constructs in plants and it would take routine experimentation to determine where in the construct the intron sequences would be inserted, as long as complementarity between the sense and antisense sequences was maintained for target gene suppression, as taught previously. It would have taken routine experimentation and design choice to alter the length of the self complementary molecules which targeted the target of interest.

One of ordinary skill in the art would have been motivated to inhibit the expression of target genes by these expressed RNAi molecules, for altering cellular phenotypes in order to study gene function, or to study the role of various target genes by comparing cellular processes in the absence or presence of these target genes' expression, or to inhibit a deleterious pathogenic gene of an invading organism in a

plant cell by inhibiting pathogenic target gene expression using this technique of gene silencing. One of ordinary skill in the art would have expected that the inclusion of intronic sequences would enhance expression construct stability because the inclusion of intronic sequences in expression constructs was routine in the art, as evidence by the inclusion of intronic sequences in commercial and other published expression constructs, at the time the invention was made.

One of ordinary skill in the art would have expected that the transformation of expression cassettes for target gene silencing in appropriate plant cells, whereby the concerted expression of both the sense and antisense fragments in appropriate target cells using appropriate promoters is obtained, leads to the formation of double stranded fragments directed to the target gene sequences in the transformed cells, and consequently interferes with the expression of the target gene, thereby producing inhibition of target gene expression, allowing a comparison of cellular phenotypes in the presence and absence of target gene inhibition, as taught previously by Flavell, Metzlaff and Stam.

For these reasons, the instant invention would have been obvious to one of skill in the art at the time of filing.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140

F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 22, 26, 42, 53, 54, 56, 58, 63-69, 85-97, 100-103, 106-110, 115-122, 127-134 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 35-38 of copending Application No. 11/841,737. Although the conflicting claims are not identical, they are not patentably distinct from each other because both claim sets are drawn to plants and eukaryotic cells comprising chimeric DNA comprising an operable promoter, transcription termination and polyadenylation region, and further comprising a nucleic acid sequence encoding an RNAi molecule. The claims of the instant invention also include the presence of an intron within the recombinant expression constructs, whereas the claims of copending Application No. 11/841,737 do not include introns in the recombinant constructs.

Although the conflicting claims are not identical, they are not patentably distinct from each other because both claim sets are drawn to plants and eukaryotic cells comprising chimeric DNA comprising an operable promoter, transcription termination

and polyadenylation region, and further comprising a nucleic acid sequence encoding an siRNA molecule.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

(10) Response to Argument

Claims 22, 26, 42, 53, 54, 56, 58, 63-69, 100-103, 109, and 115-122 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention for the reasons of record set forth in the Office action mailed 12-8-09.

Applicant's arguments filed 4-8-10 have been fully considered but they are not persuasive. Applicants argue that the fundamentally general nature of the instantly claimed invention has not been appropriately considered in determining whether adequate written description has been provided, and that the instant specification is more than sufficient to demonstrate that the inventors were in possession of the genus of tools claimed. Applicant also asserts that the instant disclosure provides not only general teachings of how to select and recombine DNA, but also specific examples of the production and use of the claimed chimeric genes. Applicant argues that no evidence has been provided that a person of ordinary skill in the art would not have recognized that the inventors had adequately described the full scope of the instantly

claimed invention at the time of filing, and have done so without relying on the prior art teachings of Fire et al, for which he received the Nobel Prize in 2006.

Applicant also argues that there is an inverse correlation between the level of skill and knowledge in the art and the specificity of disclosure necessary to satisfy the written description requirement. According to Applicant, one of ordinary skill in the art would immediately recognize the constructs with all their limitations and know how to make and use the constructs and cells, which can be assembled from a wide variety of freely available and well known nucleic acid sequence components. In addition, Applicant has supplied numerous post-filing date articles in support of their arguments that written description was adequately provided at the time of filing.

It must be stated at the onset that the instant rejection, however, is directed to eukaryotic cells and plants comprising chimeric nucleic acids comprising siRNA (a.k.a. RNAi) constructs that comprise at least 20 nucleotides that are fully complementary and target a nucleic acid capable of being phenotypically expressed. There is no teaching, prior to Fire's disclosure, of the inhibition of target gene expression using double stranded inhibitory constructs of less than approximately 550 base pairs. Applicants did not have support, prior to Fire's disclosure, for the limitations instantly claimed, particularly with respect to siRNA of lengths as short as 20 nucleotides in length per strand.

Fire and Mello received the Nobel Prize in Physiology or Medicine in 2006 for their discovery of the fundamental mechanism for controlling the flow of genetic information. Pertinent parts of the Press Release are quoted here to illustrate that,

while Applicants' can reasonably assert that they had support for what was specifically reduced to practice, as described below, comprising fully complementary pair constructs of at least 558 nucleotides in length, Applicants did not have possession of siRNA constructs comprising strands of at least 20 nucleotides in length. Nor was the mechanism of gene silencing using short siRNA molecules delineated until Fire and Mello had elucidated this silencing mechanism using short dsRNA molecules.

The press release describes the chronology of elucidation of siRNA gene silencing, for which Fire and Mello were awarded the Nobel Prize: "After a series of simple but elegant experiments, Fire and Mello deduced that double-stranded RNA can silence genes…"

> **Fire and Mello published their findings in the journal Nature on February 19, 1998. Their discovery clarified many confusing and contradictory experimental observations and revealed a natural mechanism for controlling the flow of genetic information.** This heralded the start of a new research field…

(emphasis added).

What's more, the press release also explicitly states that "The components of the RNAi machinery were identified during the **following years**." Also made clear in announcing the Nobel Prize was the fact that the mechanism causing petunias to change their color, had "remained enigmatic" until the work of Fire and Mello. It is therefore unclear how Applicants can state that they had proper support for the limitations instantly claimed, and by which they now seek to predate Fire's work, particularly using short siRNA molecules as instantly claimed.

The instant specification teaches fully complementary pair constructs for reducing the phenotypic expression of a transgenic Gus gene (of approximately **1580 base pairs**), and complementary pair constructs for reducing the phenotypic expression of the Δ12 desaturase target gene in *Arabidopsis* (of approximately **620 base pairs**) which complementary pair constructs additionally comprise the pyruvate orthophosphate dikinase 2 intron 2 from *Flaveria trinervia* (SEQ ID NO. 7) in forward or reverse orientation. The declarations filed 5-1-08 teach three expression constructs, one which comprises a double stranded hairpin of at least **750 base pairs** that comprises a sequence fully complementary to and targets the potato virus Y gene, and which expression construct further comprises intron 2 of pdkA, which was transformed into *Agrobacterium*, and a second and third double stranded construct of at least **558 base pairs** that targets and is fully complementary to the target GUS gene, and optionally comprise the Ubi-1 intron, which nucleic acid constructs were transformed into rice calli.

Contrary to Applicant's assertions, written description is determined based on what was in Applicant's possession at the time of filing. In this regard, the post-filing publications do not supplement what was missing in the originally filed disclosure. Applicants, in their response filed 5-1-08, provided declarations describing experiments that were performed prior to December 23, 1997, the priority date of the Fire patent, USPN 6,506,559. These declarations were submitted in an attempt in turn to remove the Fire patent as prior art in addressing the obviousness rejection of record. Applicant simply cannot have it both ways. But one cannot successfully argue on the one hand

that the concrete examples provided at or before the time of filing would lead one of skill in the art to make and use the full scope of the instantly claimed invention without undue experimentation – and in the absence of any concrete examples of using constructs of less than 550 nucleotides in length - while, on the other hand, argue that without Fire's contribution, any person of ordinary skill in the art would be lead to the instantly claimed invention by relying solely on Applicant's teachings.

See also page 3 of the declaration of Marc De Block submitted on 6-8-07, paragraph 14, reporting hairpin constructs that failed to provide a predictable phenotype of differences in flowering in oilseed rape, and depended on the insertion of a particular intron in a particular expression construct. The declarations and experimental examples provided in the initial disclosure failed to teach the successful inhibition of RNAi molecules with size ranges of less than approximately 550 nucleotides per targeting strand. No examples of less than 550 nucleotides per targeting strand were provided by Applicant.

The examples provided in the declarations and in the instant disclosure are not representative of the very broad genus of compounds, eukaryotic cells, and plants claimed, which encompass any plant and any eukaryotic cell comprising any nucleic acid capable of expressing a phenotype, and comprising chimeric DNA comprising an operable promoter, transcription termination and polyadenylation region, and further comprising any DNA region encoding a region capable of forming an artificial hairpin RNA structure with a double stranded RNA stem by base pairing between regions with a sense and an antisense nucleotide sequence, which sense nucleotide sequence

includes at least 20, 50, 100, or 550 consecutive nucleotides having 100% sequence identity with at least 20, 50, 100, or 550 consecutive nucleotides of any nucleic acid of interest, and which antisense sequence includes at least 20, 50, 100, or 550 consecutive nucleotides having 100% sequence identity with said at least 20, 50, 100, or 550 consecutive nucleotides of the sense sequence, and which chimeric DNA further comprises an intron.

Applicants, by providing the examples described above, were not in possession of the broad array of compounds, cells, or plants claimed, comprising any nucleic acid capable of expressing a phenotype, and further comprising a hairpin construct fully complementary and targeting any nucleic acid capable of expressing a phenotype, at the time of filing. The genus of plants, eukaryotic cells and chimeric constructs is expansive, and adequate representation of species encompassing this myriad of plants, eukaryotic cells and chimeric constructs has not been made, either prior to December 23, 1997, or at the time of filing.

The specification fails to teach or adequately describe a representative number of species in the genus such that the common attributes or characteristics concisely identifying members of the proposed genus are exemplified (e.g. the myriad of sequences encompassed by the genus intron, or intronic sequences is vast, and further whereby any intronic sequence is inserted anywhere within the DNA construct and a DNA chimeric construct generates a gene silencing construct which reduces the phenotypic expression of any nucleic acid of interest in any eukaryotic cell). And because the genus claimed is so highly variant, the description provided is insufficient

whereby a representative number of chimeric constructs provide for the functions claimed, of reducing the phenotypic expression of any nucleic acid of interest in any eukaryotic cell or plant. One of skill in the art would reasonably conclude that the disclosure, at the time of filing, fails to provide a representative number of species to describe the broad genus claimed.

Applicant has not provided adequate support for any of their stated assumptions. Applicant has provided very limited examples of experimental data which pre-date Fire, but in no way provide support to pre-date Fire's pioneering work for which he received the Nobel Prize. The instant disclosure relies on hindsight reasoning gained from Fire's teachings in an attempt to remove these teachings as prior art. For this reason, Applicant's arguments fail. Contrary to Applicant's assertions, neither the declarations filed on 5-1-08, nor the teachings in the instant disclosure provide adequate support for written description of the broadly claimed invention.

Claims 22, 26, 42, 53, 54, 56, 58, 63-69, 100-103, 109, 115-122 are rejected under 35 U.S.C. 103(a) as being unpatentable over Fire et al (USPN 6,506,559) in view of Brown et al (USPN 5,859,347), Lusky et al (USPN 6,350,575) and Schiedner et al (Nature: Genetics, Vol. 18, pages 180-183, 1998), the combination in view of Baracchini et al (USPN 5,801,154) for the reasons of record set forth in the Office action mailed 12-8-09.

Applicant's arguments filed 4-8-10 have been fully considered but they are not persuasive. Applicant argues that the instant invention would not have been obvious to

one of skill in the art for several reasons. Applicant argues on the one hand that anyone of skill in the art would have realized that Applicant was in full possession of the very broad genus of plants, eukaryotic cells and constructs claimed, either prior to the time of filing (e.g. predating December 23, 1997) or at the time of filing the instant disclosure. On the other hand, Applicant argues that no one would have found the instant invention obvious at the time of filing the instant disclosure (i.e. after the filing of Fire priority documents for USPN 6,506,559, or prior to December 23, 1997).

Applicant argues that Fire does not qualify as prior art because sufficient evidence has been provided by Declarants on 5-1-08 that the instantly claimed invention was reduced to practice for the complete embodiments prior 12-23-97, the priority date of Fire.

Contrary to Applicant's assertions, Applicant has not provided adequate support for any of their stated assumptions as argued in the written description rejection above, and reiterated here. Applicant has provided very limited examples of experimental data which pre-date Fire, but in no way provide support to pre-date Fire's pioneering work for which he received the Nobel Prize. The instant disclosure relies on hindsight reasoning gained from Fire's teachings in an attempt to remove these teachings as prior art. For this reason, Applicant's arguments fail. Contrary to Applicant's assertions, neither the declarations filed on 5-1-08, nor the teachings in the instant disclosure provide adequate support for written description of the broadly claimed invention.

There is no teaching, prior to Fire's disclosure, of the inhibition of target gene expression using double stranded inhibitory constructs of less than approximately 550

base pairs. Applicants did not have support, prior to Fire's disclosure, for the limitations instantly claimed, particularly with respect to siRNA of lengths as short as 20 nucleotides in length per strand.

Fire and Mello received the Nobel Prize in Physiology or Medicine in 2006 for their discovery of the fundamental mechanism for controlling the flow of genetic information. Pertinent parts of the Press Release are quoted here to illustrate that, while Applicants' can reasonably assert that they had support for what was specifically reduced to practice, as described below, comprising fully complementary pair constructs of at least 558 nucleotides in length, Applicants did not have possession of siRNA constructs comprising strands of at least 20 nucleotides in length. Nor was the mechanism of gene silencing using short siRNA molecules delineated until Fire and Mello had elucidated this silencing mechanism using short dsRNA molecules.

The press release describes the chronology of elucidation of siRNA gene silencing, for which Fire and Mello were awarded the Nobel Prize: "After a series of simple but elegant experiments, Fire and Mello deduced that double-stranded RNA can silence genes…"

> **Fire and Mello published their findings in the journal Nature on February 19, 1998. Their discovery clarified many confusing and contradictory experimental observations and revealed a natural mechanism for controlling the flow of genetic information.** This heralded the start of a new research field…

(emphasis added).

What's more, the press release also explicitly states that "The components of the RNAi machinery were identified during the **following years**." Also made clear in

announcing the Nobel Prize was the fact that the mechanism causing petunias to change their color, had "remained enigmatic" until the work of Fire and Mello. It is therefore unclear how Applicants can state that they had proper support for the limitations instantly claimed, and by which they now seek to predate Fire's work, particularly using short siRNA molecules as instantly claimed.

The instant specification teaches fully complementary pair constructs for reducing the phenotypic expression of a transgenic Gus gene (of approximately **1580 base pairs**), and complementary pair constructs for reducing the phenotypic expression of the Δ12 desaturase target gene in *Arabidopsis* (of approximately **620 base pairs**) which complementary pair constructs additionally comprise the pyruvate orthophosphate dikinase 2 intron 2 from *Flaveria trinervia* (SEQ ID NO. 7) in forward or reverse orientation. The declarations filed 5-1-08 teach three expression constructs, one which comprises a double stranded hairpin of at least **750 base pairs** that comprises a sequence fully complementary to and targets the potato virus Y gene, and which expression construct further comprises intron 2 of pdkA, which was transformed into *Agrobacterium*, and a second and third double stranded construct of at least **558 base pairs** that targets and is fully complementary to the target GUS gene, and optionally comprise the Ubi-1 intron, which nucleic acid constructs were transformed into rice calli.

Applicant argues that Fire, if used as prior art, mentions introns only within the context of target regions to be silenced by his siRNA constructs and that no inhibition was obtained in attempts to target introns using siRNA constructs. Applicant argues

that Brown is also improperly relied upon because Brown specifically directed inclusion of particular intron sequence into a non-translated leader of a chimeric protein expression gene and the presently claimed chimeric constructs are not directed to protein expression constructs. Applicants argue further that, even if one might speculate that increased protein expression of Brown was related to increased transcription of RNA, there is no indication in Fire that increased transcription of a chimeric gene encoding a dsRNA molecule would lead to increased silencing of the target gene and that lower concentrations of RNA molecules were found effective for dsRNA mediated gene silencing. Applicant also argues the teachings of Lusky and Schnieder are not relevant because they provide no reason for modifying a chimeric gene encoding an artificial hairpin dsDNA to include an intron, And, according to Applicant, the inclusion of intronic sequences into the recombinant expression constructs encoding siRNA would have no apparent relevance to improving dsRNA silencing.

Contrary to Applicant's assertions, Fire mentions repeatedly the ability to express siRNA as a self-folding, double stranded molecule from an appropriate expression construct, or as separate strands. As for a hairpin structure between the sense and antisense strands of the siRNA, Fire explicitly teaches in lines 43-47 of col. 4 (emphasis added):

> The double-stranded structure may be formed **by a single self-complementary RNA strand or two complementary RNA strands**. RNA duplex formation may be initiated either inside or outside the cell…

Fire reiterates this in col. 7, lines 42-45. In addition, in col. 13, lines 61-63, Fire teaches that such siRNA constructs may include "the RNA molecule itself, an **expression construct** capable of expression of the RNA, or organisms transfected with the expression construct." And, regarding the components to be optionally included within a recombinant expression construct for expressing the siRNA, Fire also explicitly teaches in the bridging paragraph, col. 8-9 (emphasis added) (citations omitted):

> RNA may be synthesized either in vivo or in vitro. Endogenous RNA polymerase of the cell may mediate transcription in vivo, or cloned RNA polymerase can be used for transcription in vivo or in vitro. For transcription from a transgene in vivo or an expression construct, a regulatory region (**e.g.,** promoter, enhancer, silencer, splice donor and acceptor, polyadenylation) may be used to transcribe the RNA strand (or strands)… The RNA strands may or may not be polyadenylated; the RNA strands may or may not be capable of being translated into a polypeptide by a cell's translational apparatus... The use and production of an expression construct are known in the art...

And, contrary to Applicant's assertions, researchers have routinely incorporated intronic sequences for enhancing stability of recombinant constructs for decades. Molecular biologists have routinely included intronic sequences within expression constructs for enhancing gene expression in plants because the use of intronic sequences for enhancing vector stability would logically enhance expression of a desired gene in cells, whether that expression construct encoded a protein or an inhibitory molecule, as evidenced by the prior art teachings of Brown and Schiedner. In addition, Lusky teaches the routine incorporation of intronic sequences in expression constructs and it was well known in the art that the inclusion of introns enhances the expression of RNA in plants. Fire teaches the general applicability of recombinant

expression systems for generating siRNA constructs in an appropriate target cell, and recombinant expression systems, including those commercially available, routinely include intronic sequences with the vector constructs. What's more, the limited success pointed out by Applicant, in Fire's limited ability to target an intron with an siRNA construct, actually provides added motivation to successfully include an intron sequence within a recombinant expression system without fear of inadvertently inhibiting intronic target sequences. This provides motivation for relying on the well known advantages of incorporating intronic sequences into recombinant expression constructs for improving recombinant construct stability and, in turn, enhancing recombinant expression, as had been done routinely in the art for decades. So, contrary to Applicant's assertions, one of skill in the art could have readily predicted that including intronic sequences in recombinant expression constructs, which sequences were well established in the prior art for enhancing stability and expression of recombinant constructs, would in the instantly claimed expression constructs also enhance stability and expression of the siRNA.

For these reasons, the instant rejection is properly maintained.

Claims 22, 26, 42, 53, 54, 56, 58, 63-69, 100-103, 109, 115-122 are rejected under 35 U.S.C. 103(a) as being unpatentable over Flavell (Proc. Natl. Acad. Sci., Vol. 91, pages 3490-3496, 1994) Metzlaff et al (Cell, Vol. 88, pages 845-854, 1997), and Stam et al (Annals of Botany, Vol. 79, pages 3-12, 1997), the combination in view of

Brown et al (USPN 5,859,347) and Lusky et al (USPN 6,350,575) for the reasons of record set forth in the Office action mailed 12-8-09.

Applicant's arguments filed 4-8-10 have been fully considered but they are not persuasive. Applicant argues that a person of ordinary skill in the art reading Flavell, Metzlaff and Stam would have found it obvious to make the dsRNA of Fire, which is contrary to the issuance of the Fire patent, and then to manufacture constructs comprising an artificial hairpin. Applicant contends that this interpretation by the Examiner is contradicted by the awarding of the Nobel Prize to Fire and Mello. In addition, according to Applicant, the invention for which Fire was awarded the Nobel Prize was actually reduced to practice by Applicant prior to Fire's discovery of siRNA.

As a point of clarification, the instant rejection does not seek to render obvious Fire's discovery of **small interfering RNA molecules, *a.k.a.* siRNA** (i.e. of approximately 25 nucleobases or more) as a more potent tool for inhibiting target gene expression compared to antisense or ribozymes (see, e.g. col. 1-3 of Fire, which explicitly compares the advantages of small interfering RNA oligonucleotides with other inhibitory oligonucleotides, such as antisense oligonucleotides. The record is clear that Applicant, in stark contrast, had constructed a few **long**, double stranded constructs and had shown that target gene inhibition was obtained. Fire, on the other hand, made the discovery that **small** antisense oligonucleotides that had been used routinely to inhibit target gene expression were actually contaminated with small, complementary strands. Once these oligonucleotides were more rigorously purified, the single stranded (antisense) oligonucleotides were compared to short, double stranded oligonucleotides

and found to be inferior in their ability to inhibit target gene expression compared to the small, double stranded versions. This simple and elegant purification experiment led to the discovery of **small, interfering RNA, siRNA** by Fire.

In contrast, Applicant logically extrapolated what was speculated in the plant literature for a decade, that long stretches of inverted repeats often coincided with plant co-suppression. That is where the teachings of Flavell, Metzlaff and Stam become relevant as prior art, in providing the forethought that lead to the next logical experiment: Will the explicit expression of these long, internal repeats (IRs) in recombinant expression constructs provide for co-suppression of a corresponding target gene, as previously observed in petunias and other plants?

Applicant argues that the publications of Flavell, Metzlaff and Stam did not contemplate that double stranded RNA structures formed between antisense RNA and the sense mRNA could be a triggering agent in gene silencing. Applicant argues that the hypotheses put forward during the mid-nineties were limited to four hypotheses: differences in methylation patterns or participating genes that interfere with transcription complex assembly, elevated competition between increased number of genes for non-diffusible sequence factors essential for transcription or translation, formation of aberrant duplexes of mRNAs and antisense RNA, and feedback inhibition due to accumulation of aberrantly high concentrations of a specific gene product in a transgenic plant, and that these four predominant hypotheses in the field essentially precluded the consideration of alternative hypotheses responsible for the co-suppression phenomena observed in plants and later in other eukaryotic cells.

Contrary to Applicant's assertions, the discussions and investigations concerning the observed phenomena of gene suppression were not limited to four hypotheses, and the references of Flavell, Metzlaff and Stam repeatedly address the limitations of these existing hypotheses, and ponder the relationship between the existence of inverted repeats as well as the possible accumulation of sense/antisense constructs and the subsequence degradation of mRNA.

For evidence of these logical speculations and extrapolations, see, *e.g.*, Flavell on p. 3490: "Degraded RNA products from both genes were found in co-suppressed fruit, suggesting that RNA transcription is not inhibited and therefore loss of mature mRNA is due to posttranscriptional turnover." (last full paragraph on p. 3490). See also the first full paragraph on p. 3491 which dispels the role of methylation in target gene inhibition: "…suppression of activity was not correlated with methylation..." And the second full paragraph on p. 3491 provides motivation to study the role of self-complementary sequences in target gene inhibition: "Ninety base pairs of homology in promoter sequences were sufficient to create a co-suppressed condition." Contrary to Applicants' assertions, investigators in the field in the mid-nineties were investigating the role of self-complementary constructs that formed aberrantly (and not excluding inverted repeats) in target gene inhibition. These self complementary constructs that resulted from the expression of inverted repeats were not limited to antisense-mRNA duplexes leading to mRNA degradation, and the presence and role of inverted repeats, particularly in observed flowering patterns in petunia, was questioned and discussed repeatedly throughout the decade preceding the Fire patent.

And, contrary to Applicants' assertions, the first paragraph on p. 3495 of Flavell invites further experimentation beyond the four proposed hypotheses:

> What is needed to evaluate the application of antisense RNA formation to the cause of down-regulation of homologous gene expression, in at least some examples of trans-inactivation by transgenes, is a much better understanding of how antisense RNA effects down-regulation of gene expression, measurements of antisense and sense RNA levels in the relevant cells and their nuclei before as well as after RNA degradation, and knowledge of the role of RNA-dependent RNA polymerase and of the ability of accumulated RNA products to feed back and interfere with transcription. It will also be important to discover the relationship between the mRNA turnover revealed by transgenes and endogenous posttranscriptional control systems that regulate mRNA turnover.

What's more, Stam repeatedly stresses the importance of the existence of inverted repeats and their role in gene inhibition: See figure 1 on p. 4 and the bridging paragraph of pages 3-4: "These T-DNAs can be arranged 'head-to-tail' as a direct repeat (DR), and 'head-to-head' or 'tail-to-tail' as an inverted repeat (IR). **Transgenes of T-DNAs that are organized as IRs often show low expression indicating that the genes are silenced to some degree**." (citations omitted, emphasis added). And on p. 8, last full paragraph: "There are at least two possibilities; the first is that a multicopy locus is prone to deliver the hypothetical aberrant RNA assumed to trigger the cytosolic RNA degradation machinery directly... One possibility is that it occurs as a result of ectopic DNA pairing between the transgene locus and the endogenous gene(s)... these transcripts may be intrinsically unstable and rapidly degraded, or may act as aberrant RNA causing the degradation of other homologous RNAs. Not all transgene loci may be able to pair ectopically with an endogenous gene. An essential property seems that they are repetitive. **Thus far, all the T-DNA loci that we have found to induce PTGS**

of *chs* contain two or more T-DNAs arranged as IRs... There are no indications that the methylation status of the endogenous genes is changed." (citations omitted, emphasis added).

And in the first paragraph on p. 9, Stam again rules out methylation as a mechanism involved in target gene inhibition involving IRs:

> Observations with IRs and DRs in Drosophila, which lacks 5-methyl-cytidine in its DNA, indicate that repeats somehow interact with each other, leading to the formation of heterochromatin… By analyzing petunia transformants carrying CaMV-35S promoter-driven *chs* sense or antisense transgenes, Jorgensen et al. (1996) showed that the pattern of *chs* silencing in flowers correlated with the repetitiveness and organization of the transgenes in these plants. The pigmentation pattern caused by single-copy transgene inserts is mostly regular (junction type) whereas that by IRs is often complex and sometimes recognizable as the 'Cossack Dancer' pattern…"

Metzlaff also discusses the role of inverted repeats in target gene inhibition: On page 845, second full paragraph of the introduction: "Petunia plants are correlated with the number of transgenes and their arrangement in the genome." Metzlaff devotes considerable thought to the relationship between self annealing sequences and resistance of those self annealing structures to degradation (see e.g. figure 5), as well as arguing that co-suppression is not merely due to the presence of high levels of chsA RNA (see first full paragraph on page. 850). The involvement of RNA-RNA pairing in RNA turnover is questioned: "Such complementarity may have been selected as a component of an RNA turnover control system by intra- or intermolecular RNA pairing." (second full paragraph from the end on page 852). And, reviewing Jorgensen's work, the role of inverted repeats in target gene suppression is questioned: "...whereas

multiple copies of transgenes, and especially **inverted repeat** copies, enhance the probability of more extensive co-suppression and, in particular, co-suppression in leaves and stems." (second last paragraph of the discussion on p. 853) (emphasis added).

It is clear that the role of inverted repeats, and other double stranded RNA structures were pondered by those looking for underlying mechanisms of target gene suppression in eukaryotes, as illustrated by the teachings of Flavell, Stam and Metzlaff. It therefore would have been obvious to design, construct and test the ability of nucleic acid constructs comprising double stranded RNA for their ability to inhibit the expression of a known target gene in plants or in eukaryotic cells in vitro at the time of the instant invention.

And, contrary to Applicant's assertions, researchers have routinely incorporated intronic sequences for enhancing stability of recombinant constructs for decades. Molecular biologists have routinely included intronic sequences within expression constructs for enhancing gene expression in plants because the use of intronic sequences for enhancing vector stability would logically enhance expression of a desired gene in cells, whether that expression construct encoded a protein or an inhibitory molecule, as evidenced by the prior art teachings of Brown. In addition, Lusky teaches the routine incorporation of intronic sequences in expression constructs and it was well known in the art that the inclusion of introns enhances the expression of RNA in plants. It is therefore no surprise that, if there is enhanced stability of recombinant constructs, there will likely be less degradation and enhanced expression. And, in the

presence of enhanced stability and expressed siRNA, these will be enhanced target gene inhibition.

For these reasons, the instant rejection is proper and is hereby maintained.

(11) Related Proceeding(s) Appendix

No decision rendered by a court or the Board is identified by the examiner in the Related Appeals and Interferences section of this examiner's answer.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

/Jane Zara/ AU 1635

6-30-10

Conferees:

/ Christopher S. F. Low /
Supervisory Patent Examiner, Art Unit 1639

/Fereydoun G. Sajjadi/
Acting Supervisory Patent Examiner, Art Unit 1635